



Development of Methods for Evaluation of Live Viral Vaccines

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Then and Now



20 Century: most important discoveries and inventions

- * Automobile
- * Aviation / Space travel
- * Electricity
- * Nuclear Energy
- * Telephone
- * Computer / Digital technologies
- * DNA / Genetic engineering
- * Blood transfusion / organ transplantation
- * Antibiotics
- * Vaccines

Live Viral Vaccines Challenge:

- * Unique biological product
- * Not a drug substance, but the organism itself



Viruses Then

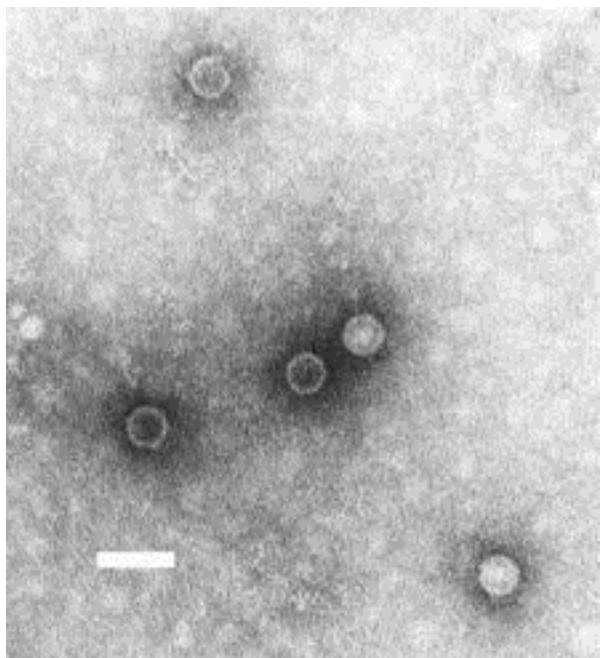
‘Contagium vivum fluidum’

Beijerinck

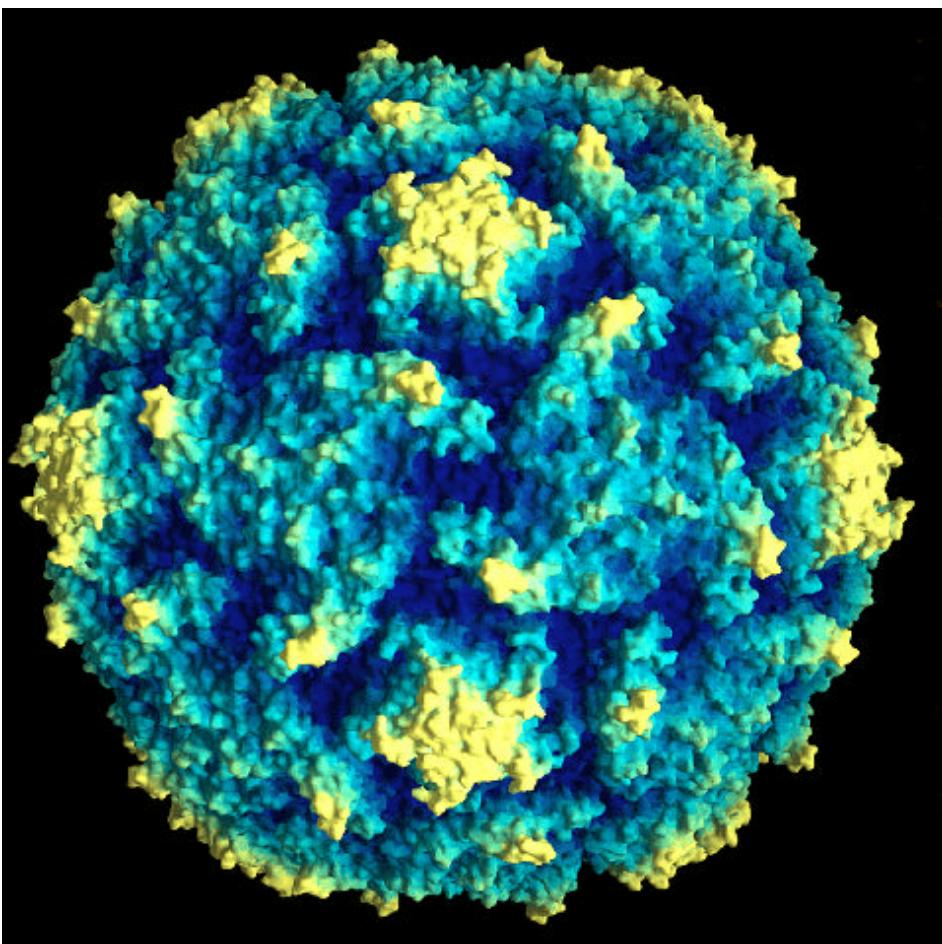
‘Every virus is a microbe’

Louis Pasteur

Viruses: view from the 1950's



Poliovirus Today





*How much do we need to know
to create safe and efficacious
vaccines?*

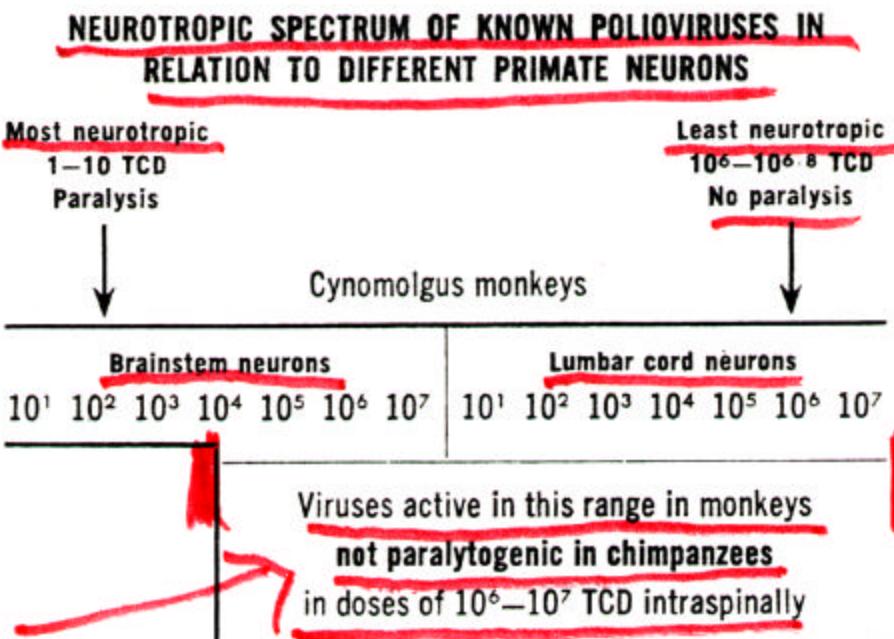


PROPERTIES OF ATTENUATED POLIOVIRUSES AND THEIR BEHAVIOR IN HUMAN BEINGS*

BY

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*Children's Hospital Research Foundation, University of Cincinnati
College of Medicine, Cincinnati, Ohio*



Monkey Neurovirulence Test



Type 1

12 + 12 monkeys

Type 2

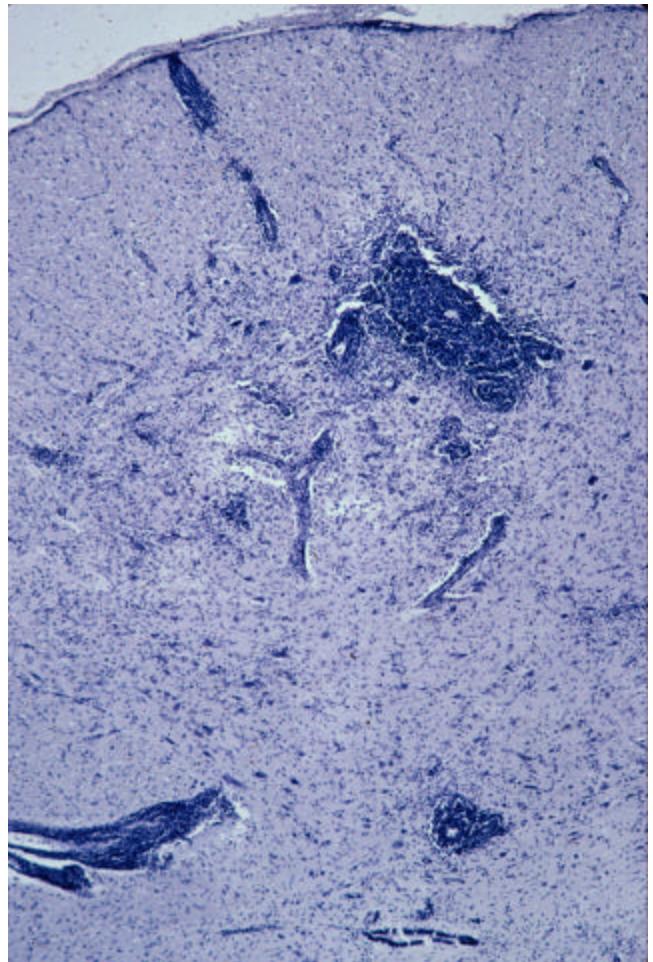
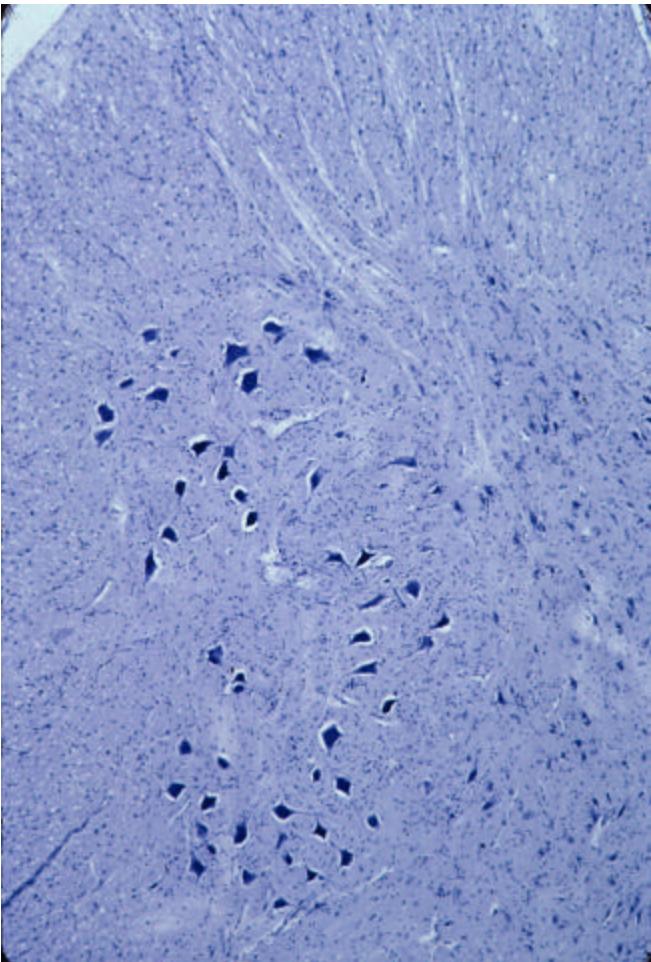
12 + 12 monkeys

Type 3

24 + 24 monkeys

Total: 96 monkeys

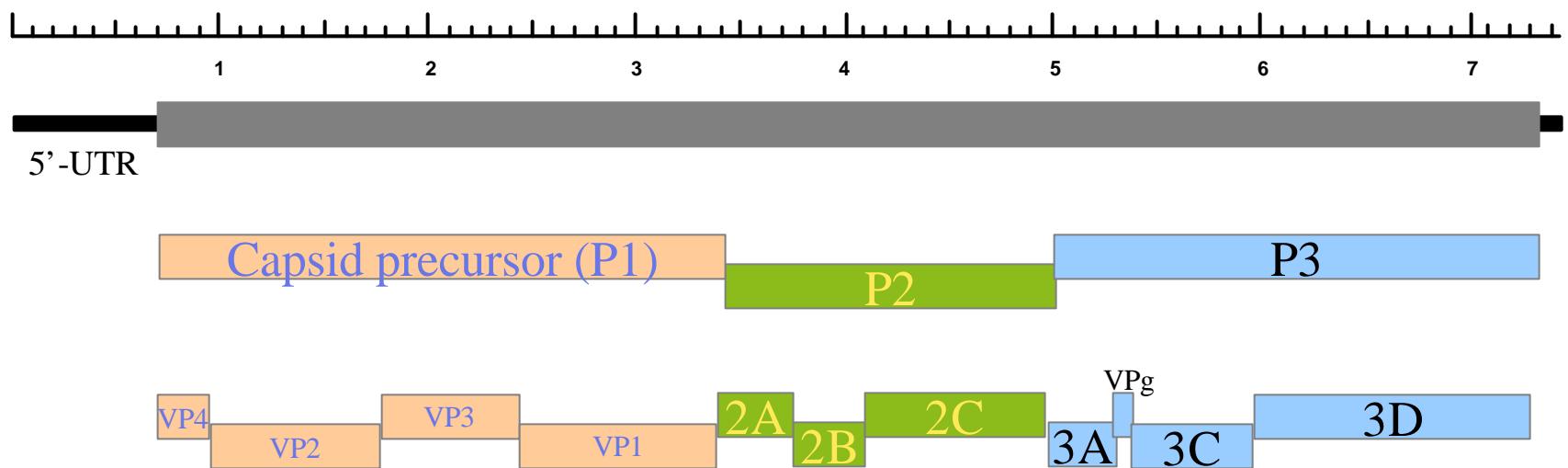
Lumbar Cord Pathology



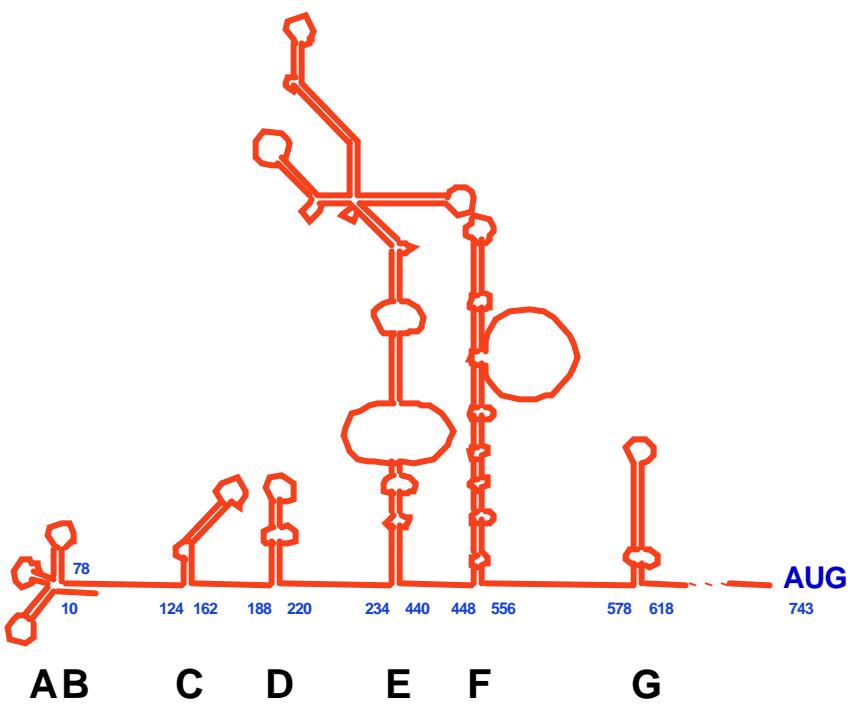
Transgenic Mouse Neurovirulence Test



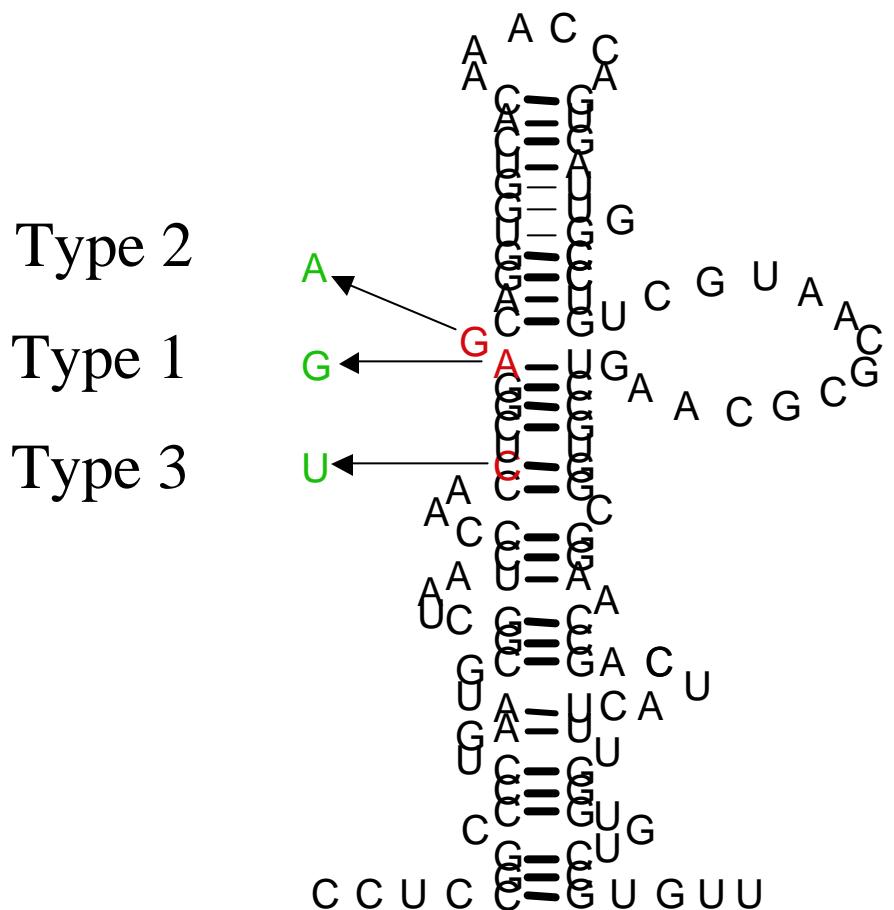
Poliovirus Genome



5'-Non-Coding Region of Poliovirus

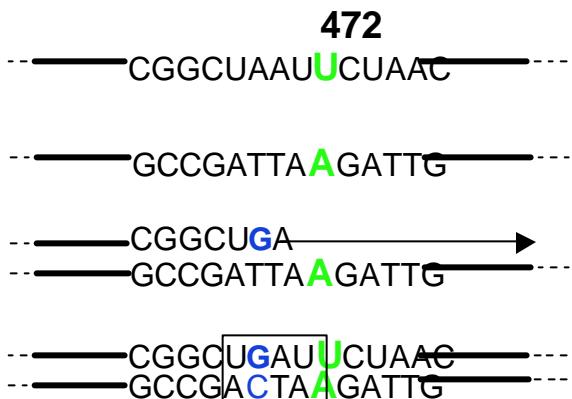


F-domain of the Polio 5'-NCR

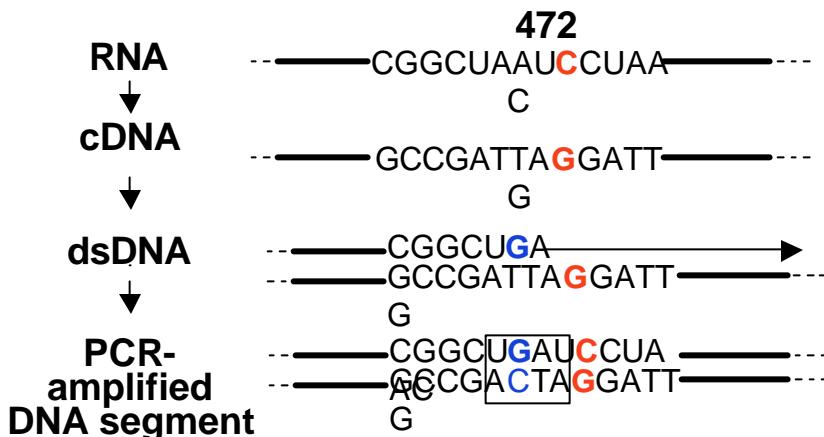


MAPREC scheme

Sabin strain



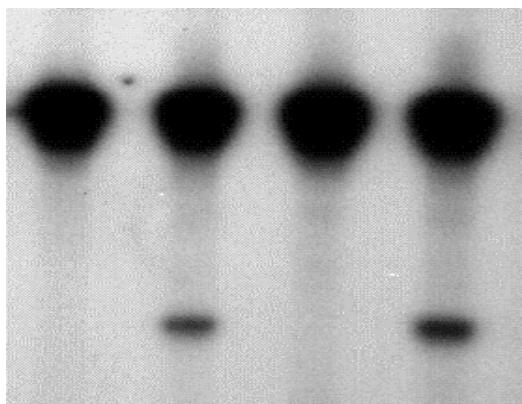
Revertant strain



GA?TC = Hinfl site

GATC = MboI site

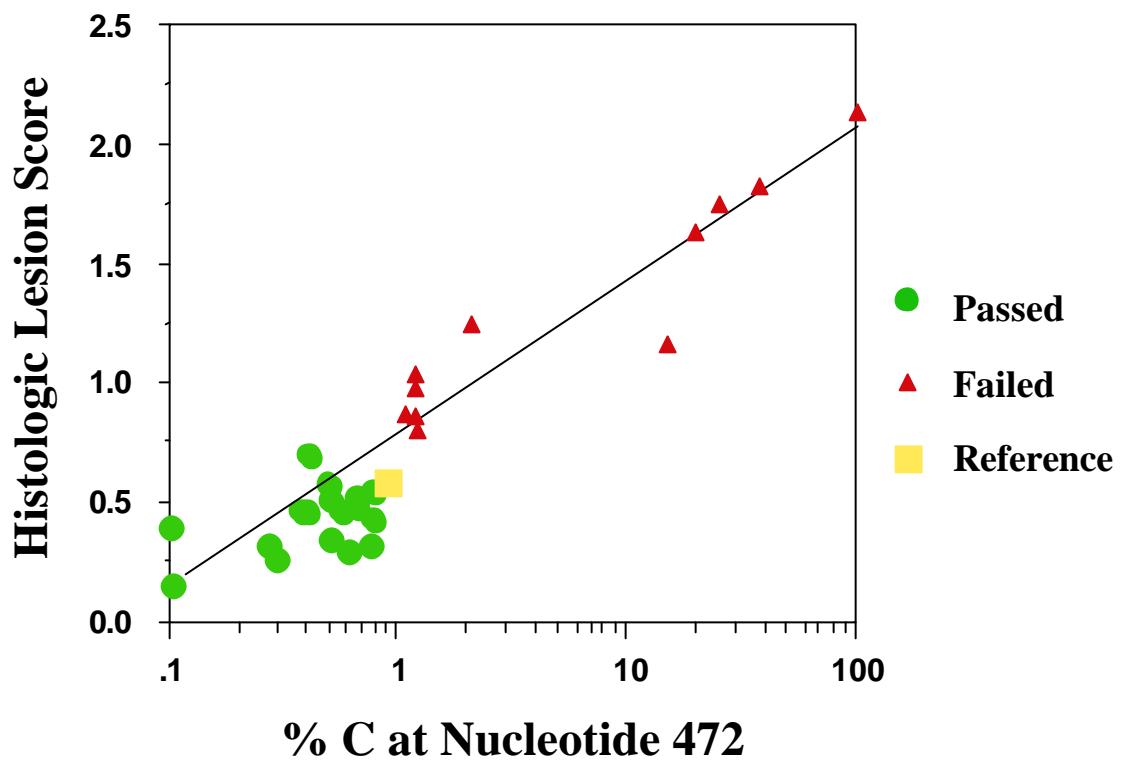
— MboI — MboI



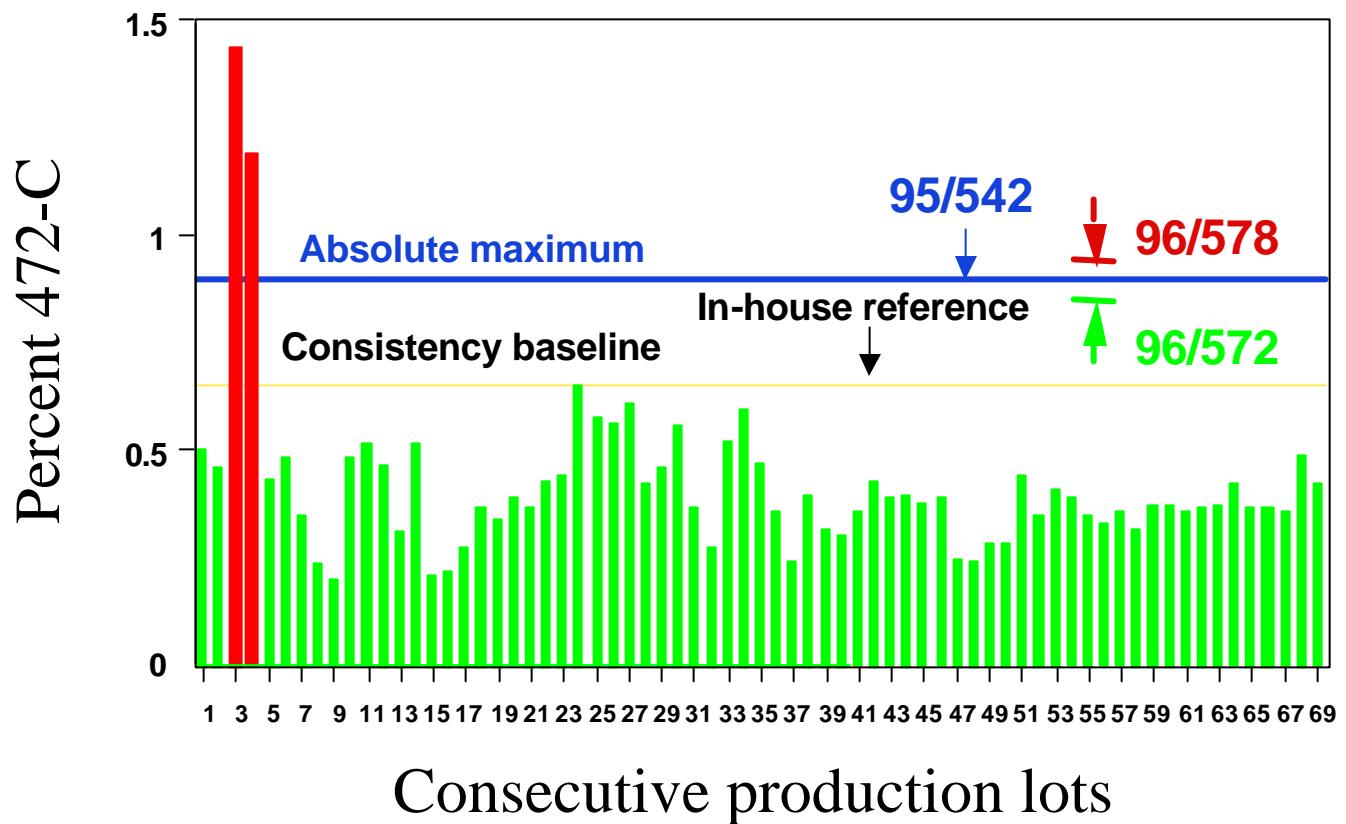
← Full-length DNA segment

← Cleavage product
indicating revertant virus

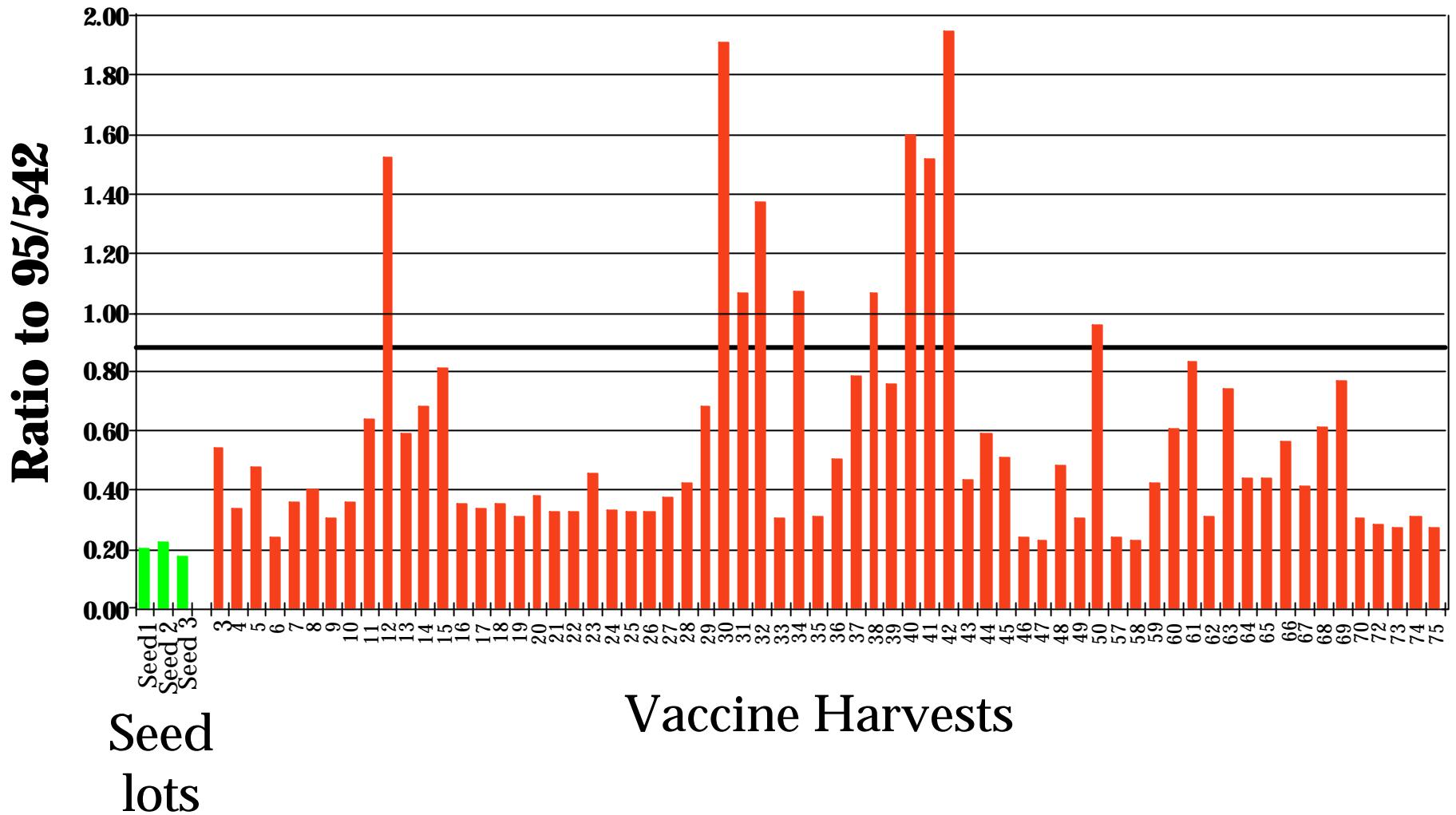
472-C Revertants Determine Lesion Score in the MNTV



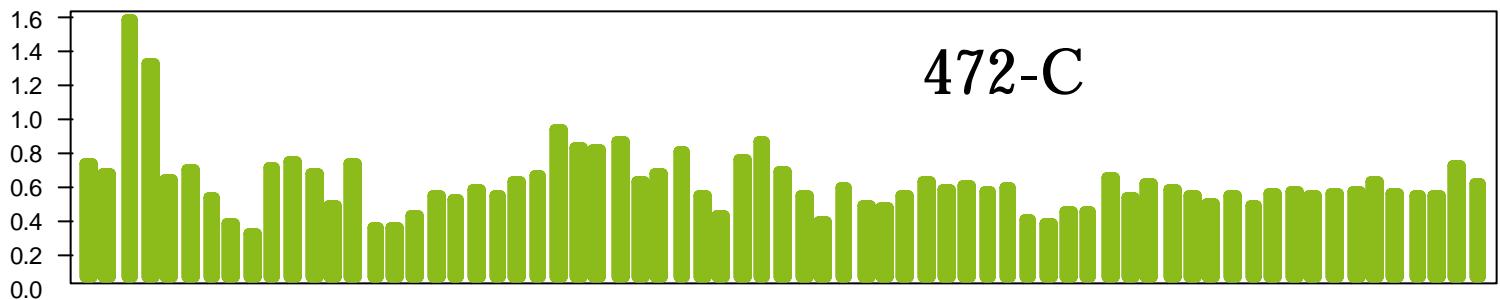
Molecular Consistency Decision



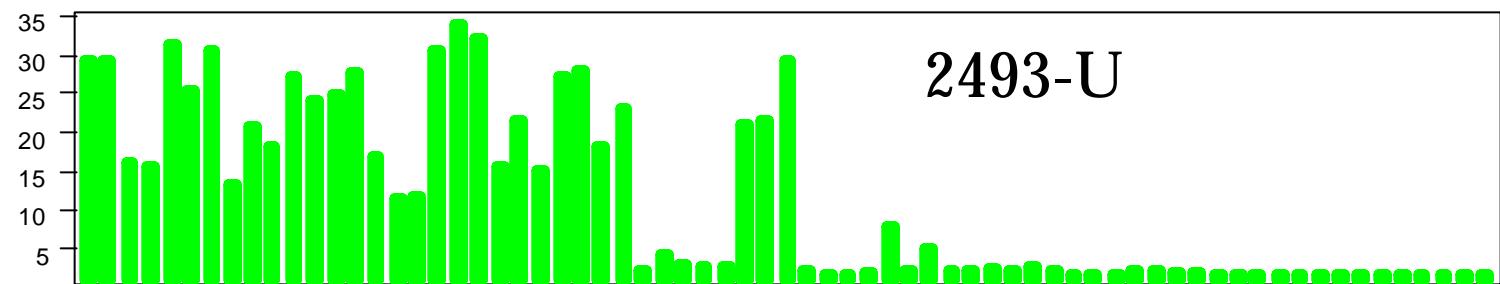
Production Consistency



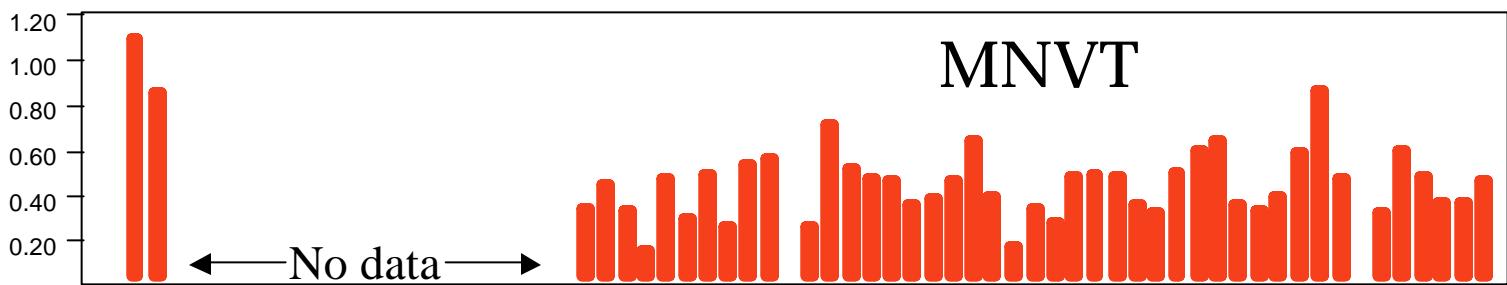
Consistency of OPV 3 Production



472-C



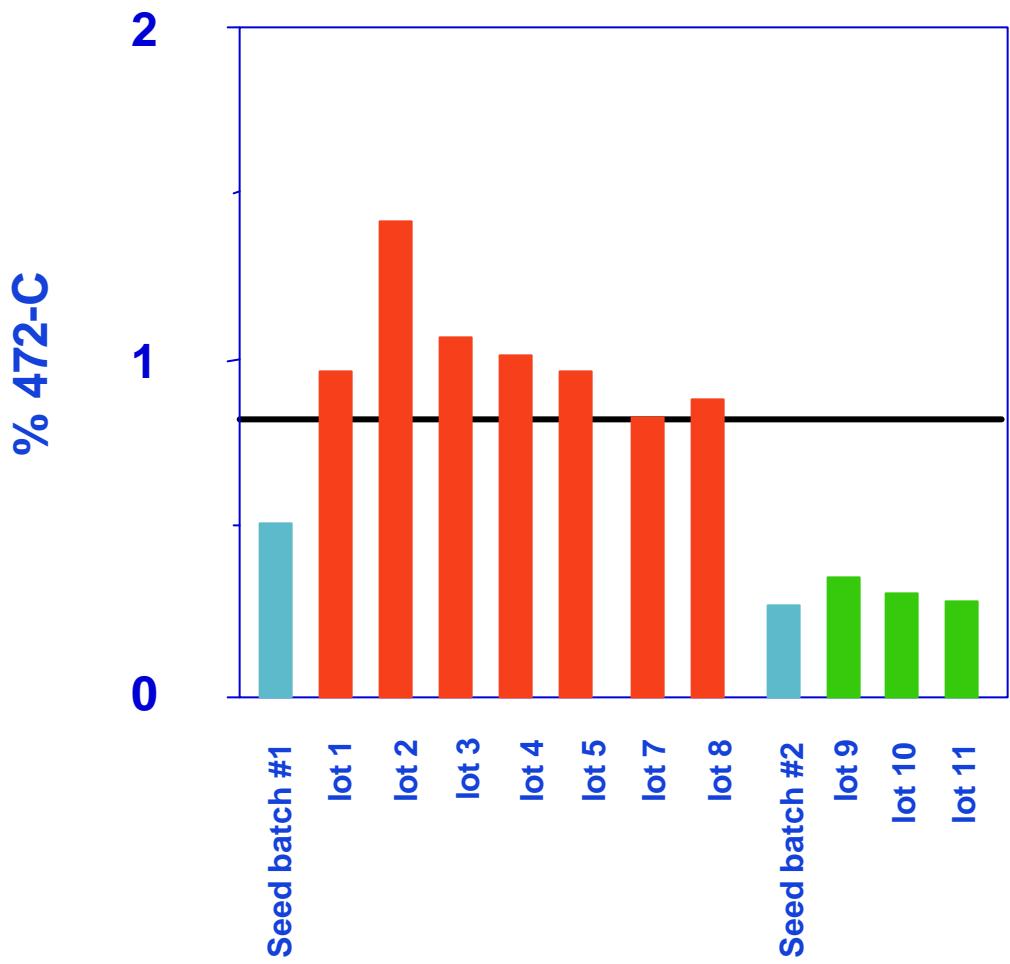
2493-U



MNVT

← No data →

MAPREC for validation of new production



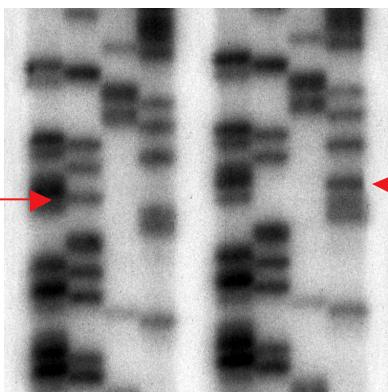
Molecular Consistency Monitoring of Live Viral Vaccines

Mutational profile of new vaccine batches should match the one for the previously manufactured product with established safety record

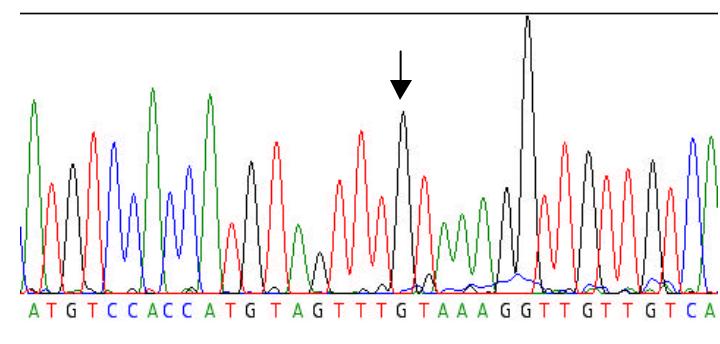
Sequence heterogeneity in YFV vaccine

Antisense

A

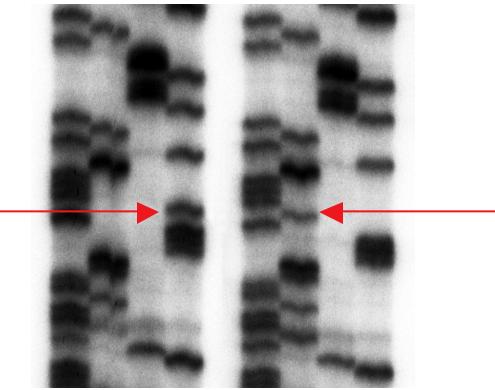


C

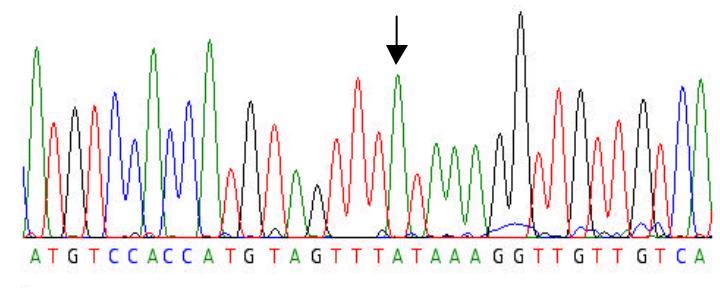


B

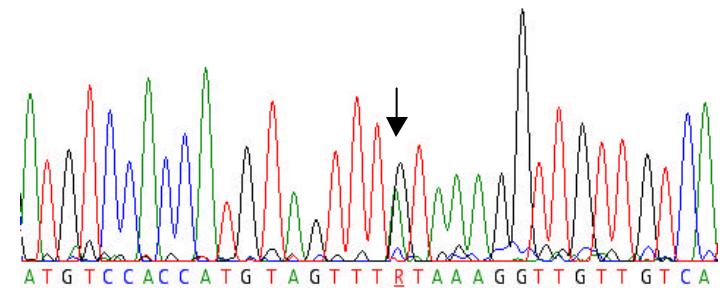
Sense



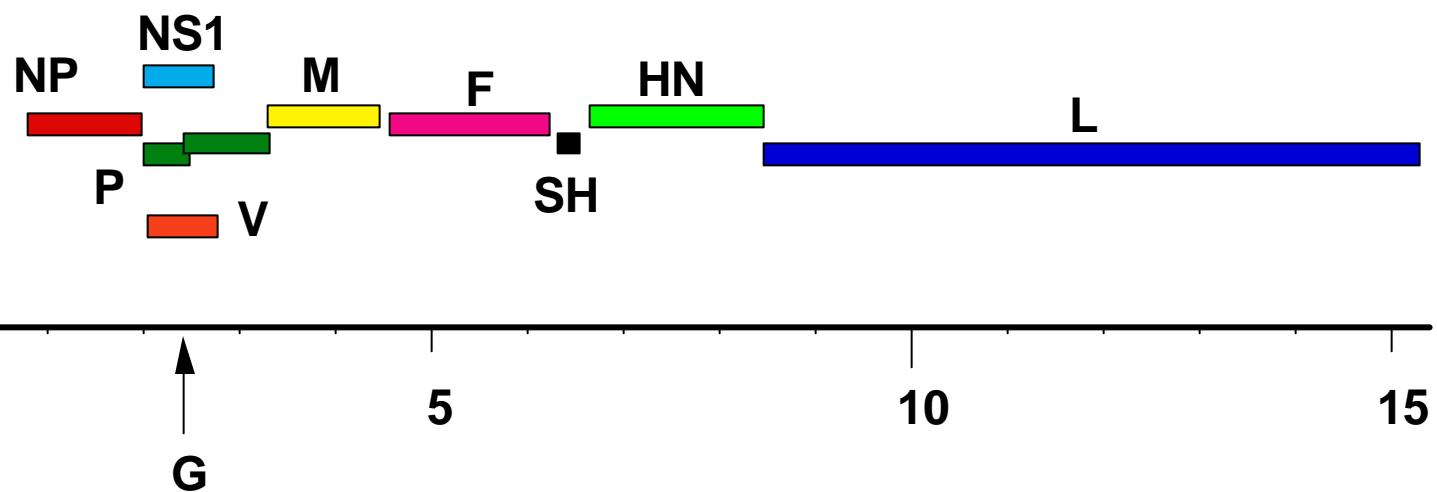
D



E



Genome of Mumps virus



Sequence Heterogeneities in Mumps Vaccines Live (Urabe)

Nucleotide	Gene	Published Sequence*	Biken	Chiron SpA	SKB	Clinical Isolates	
						1004	1005
1880	NP	C	C	C	C	C	T
6385	HN	C	C	C	C	C	A
7409	HN	A	G	G	G	G	G
7573	HN	T	T	T	T	T	C
7616	HN	A Lys	A Lys	A Lys	A/G Lys/Glu	A Lys	A Lys
8005	HN	C Asn	C Asn	C Asn	A/C Lys/Asn	C Asn	A Lys
8015	HN	G Glu	G Glu	A/G Lys/Glu	A/G Lys/Glu	G Glu	G Glu
9972	L	T Phe	T Phe	C/T Ser/Phe	C/T Ser/Phe	C Ser	T Phe
10529	L	C Pro	C Pro	C Pro	C/T Pro/Leu	C Pro	T Leu
11692	L	G Leu	G Leu	G Leu	G/T Leu/Phe	G Leu	T Phe
14049	L	C	C	C	C	C	T
15328	UTR	G	G	G	G	A	G

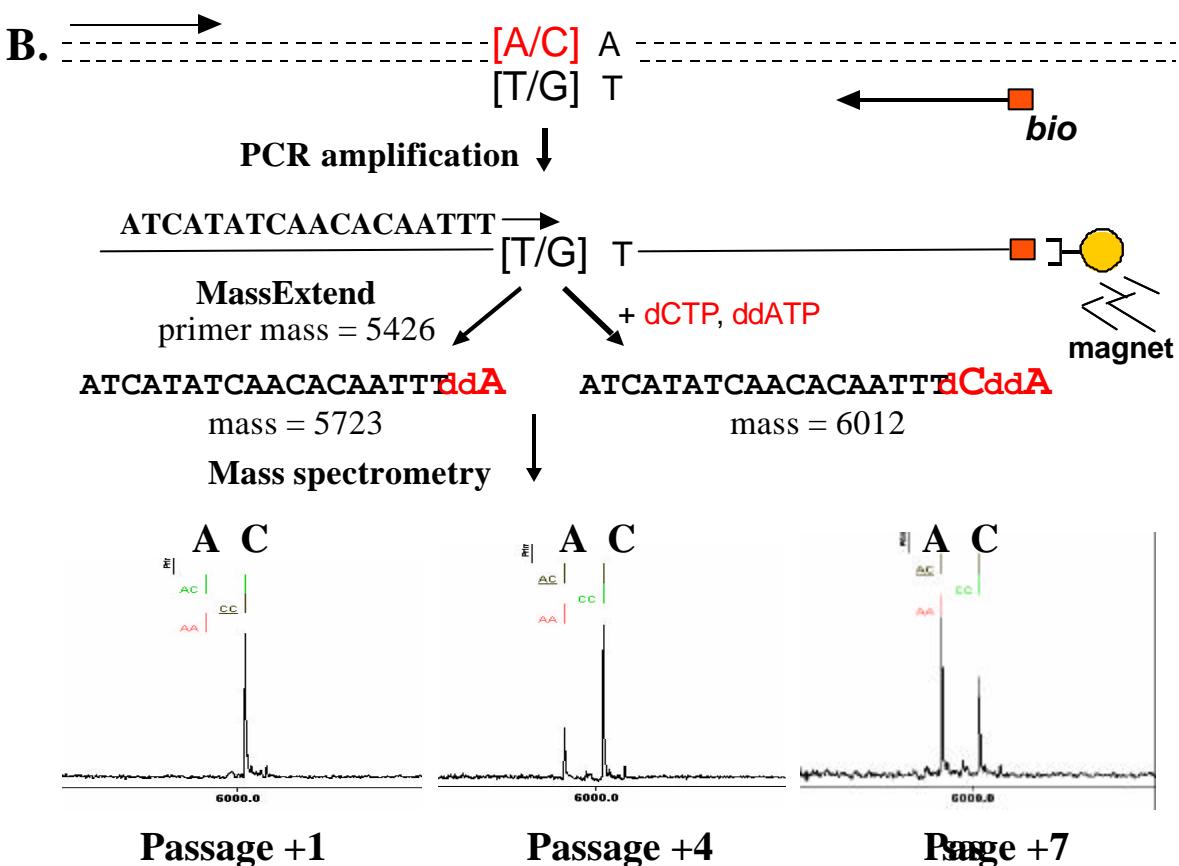
Genotyping by MALDI-TOF Mass-Spectrometry

AGTTCCGA**C**TTCGATTCTTAGGCTATT
GAA^A**G**CTATGAAATCCGATAA
 ^

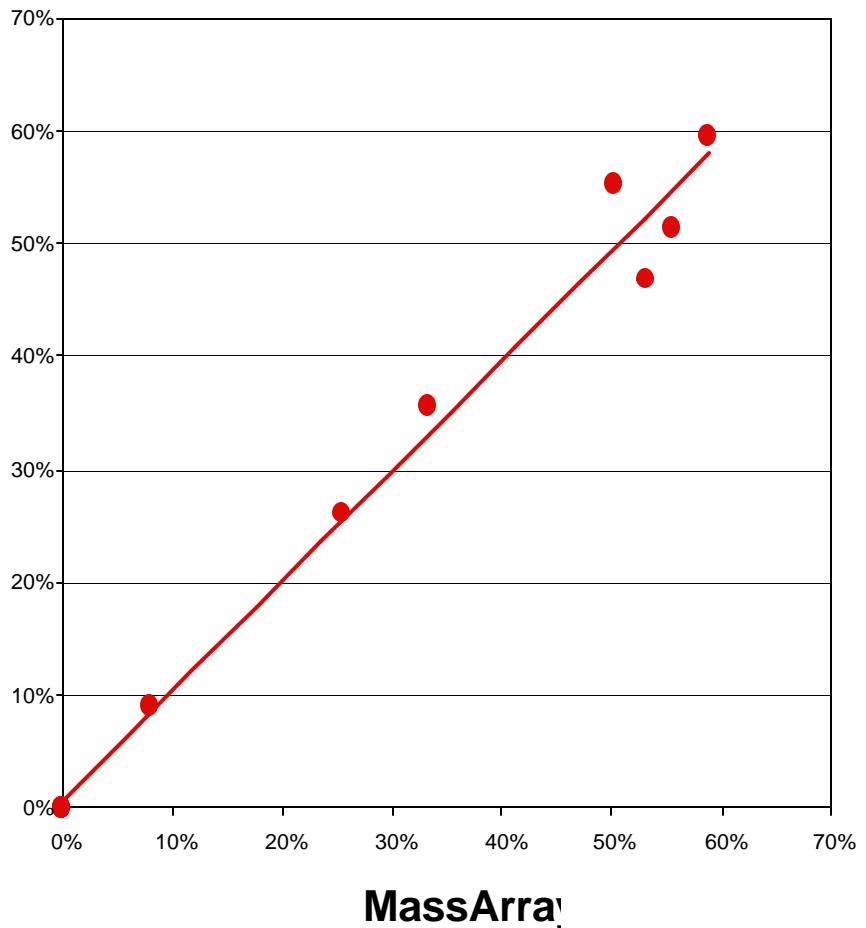
AGTTCCGA**T**TTCGATTCTTAGGCTATT
GCTAAAGCTATGAAATCCGATAA
 ^

dATP, dCTP, **ddGTP**, TTP + DNA polymerase

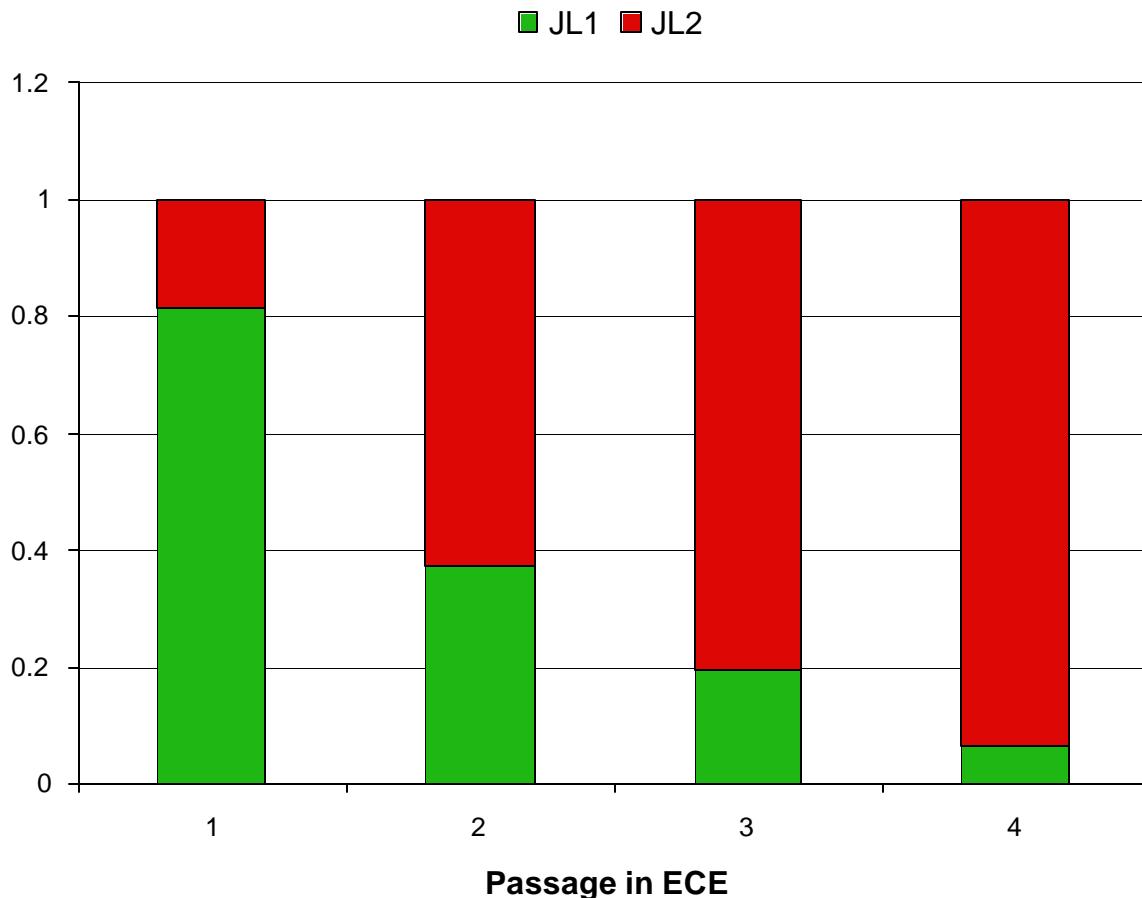
MassArray analysis of MVL



MAPREC vs. Mass-spectrometry



Selection of JL2 during virus growth in chicken embryos





*Vaccine-associated paralytic
polio cases were caused by
acceptable vaccine lots*

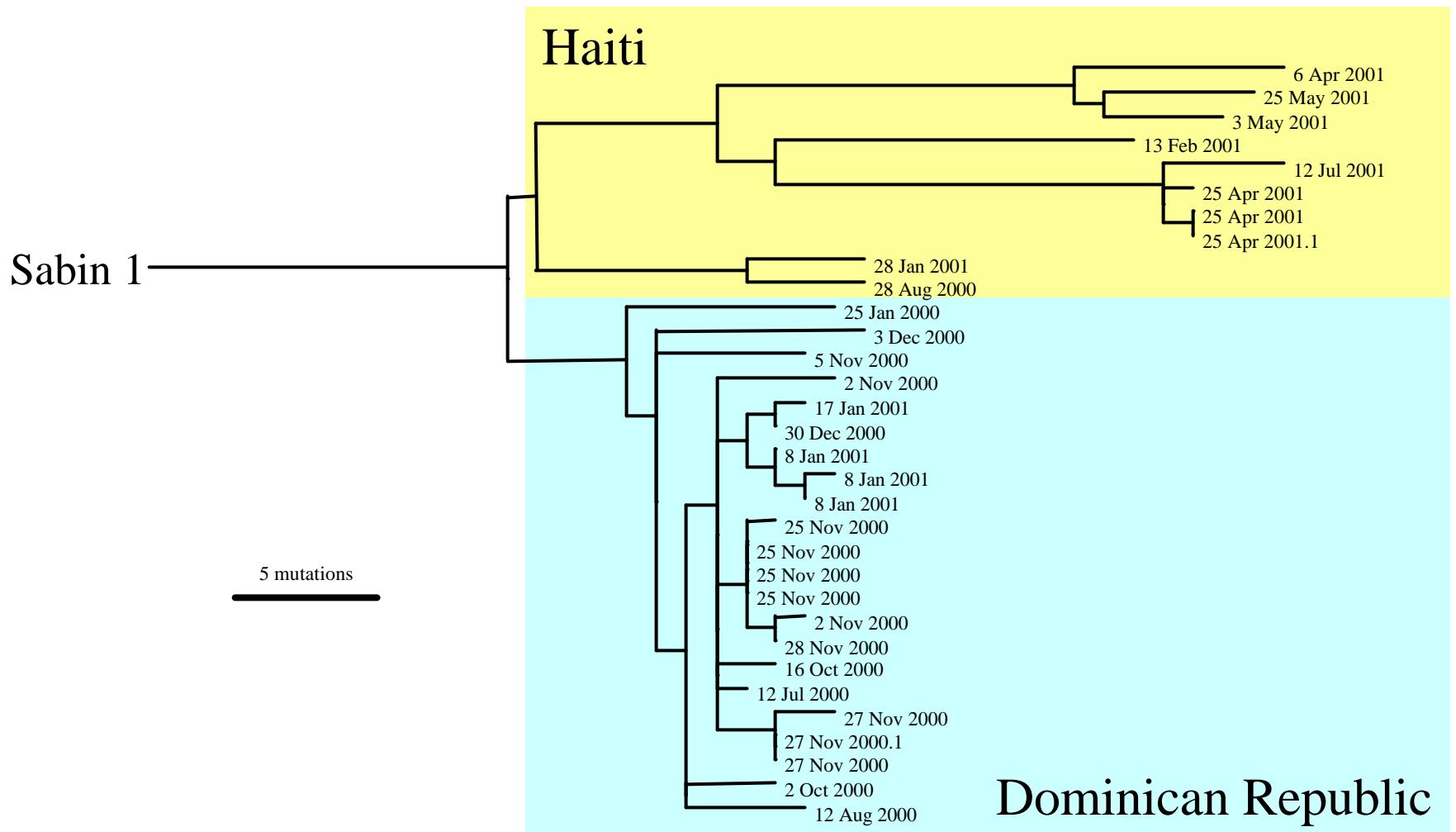
(Retrospective analysis of more than 900 lots)

Host determinants and post-vaccination events are the primary factors affecting safety of vaccines

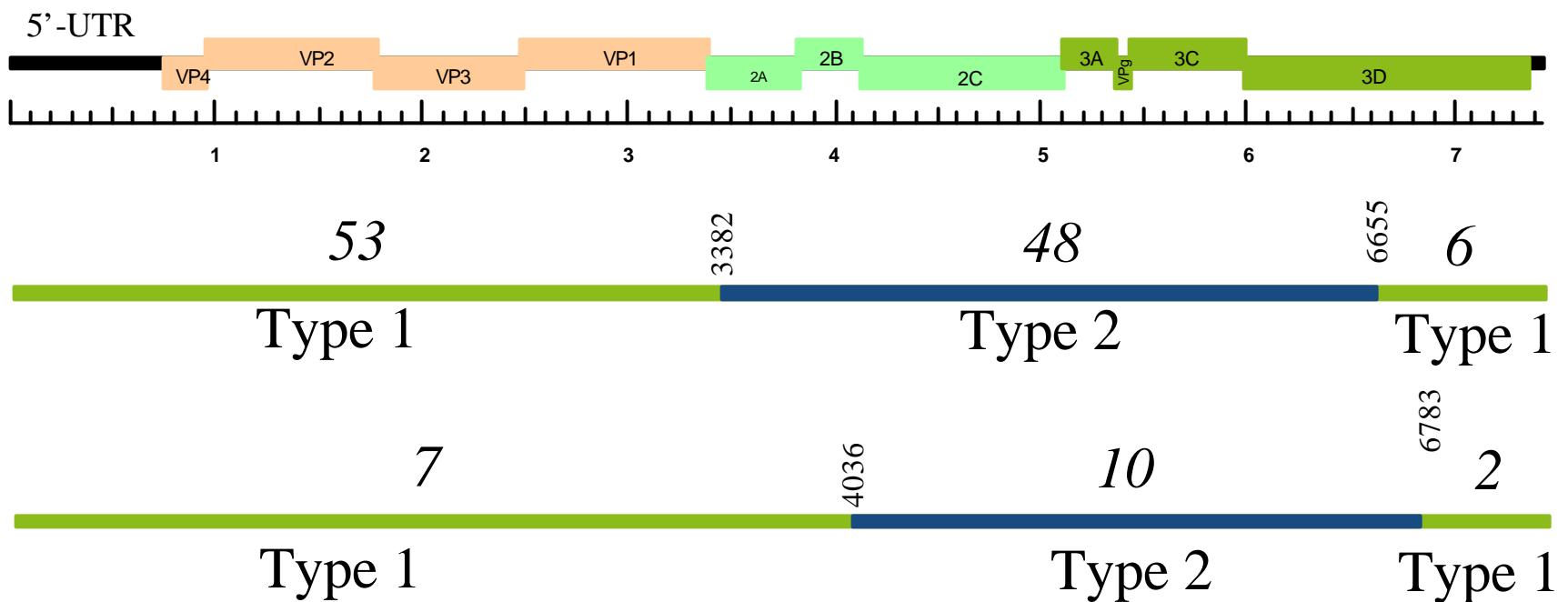
“Runaway” viruses: Circulation of VDPV

- * Vaccine-derived viruses do not necessarily fade away from environment as previously thought
- * VDPV establish sustainable chains of transmission and can cause outbreaks
- * We urgently need to understand the reasons for de-attenuation of circulating vaccine strains

Polio Outbreak in Hispaniola

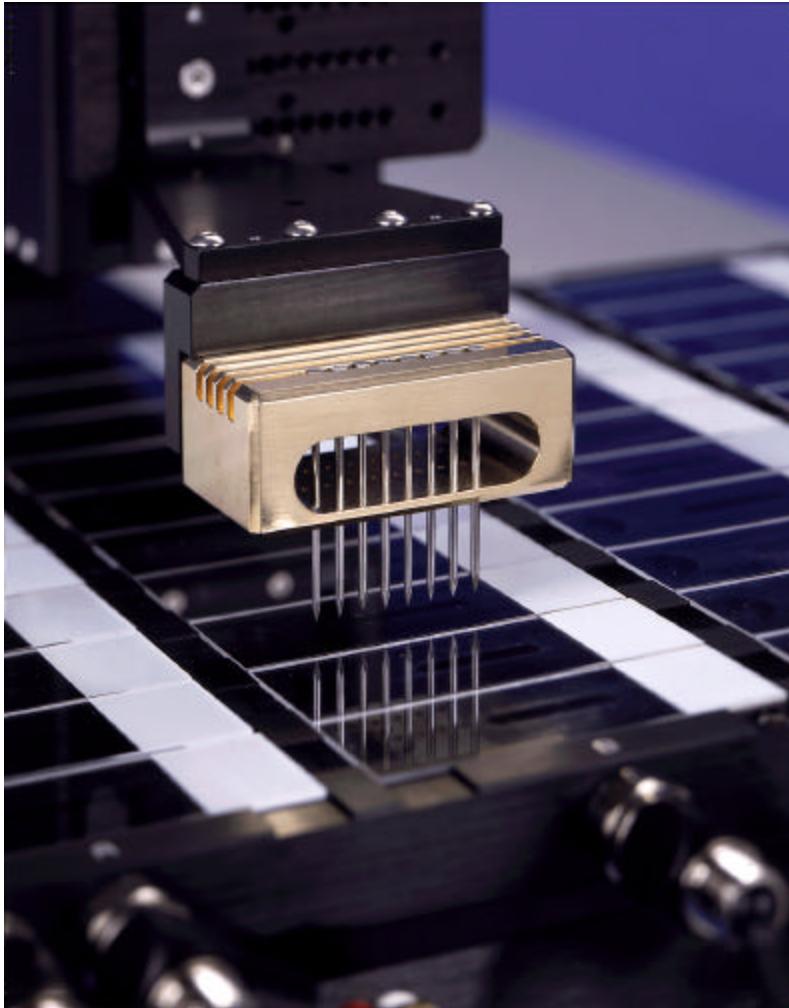


Recombinant Strains of Vaccine-derived Poliovirus

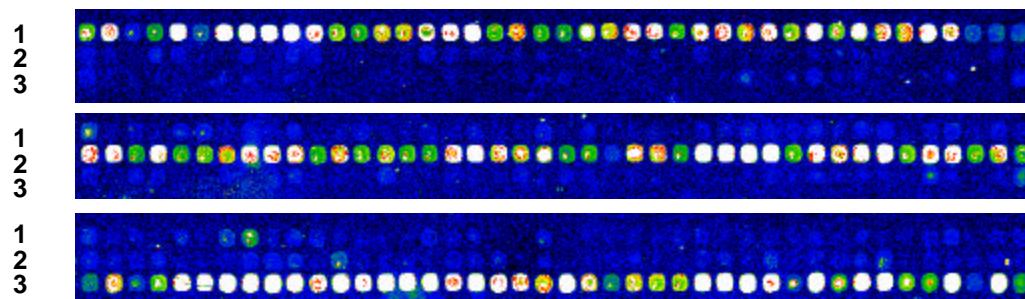
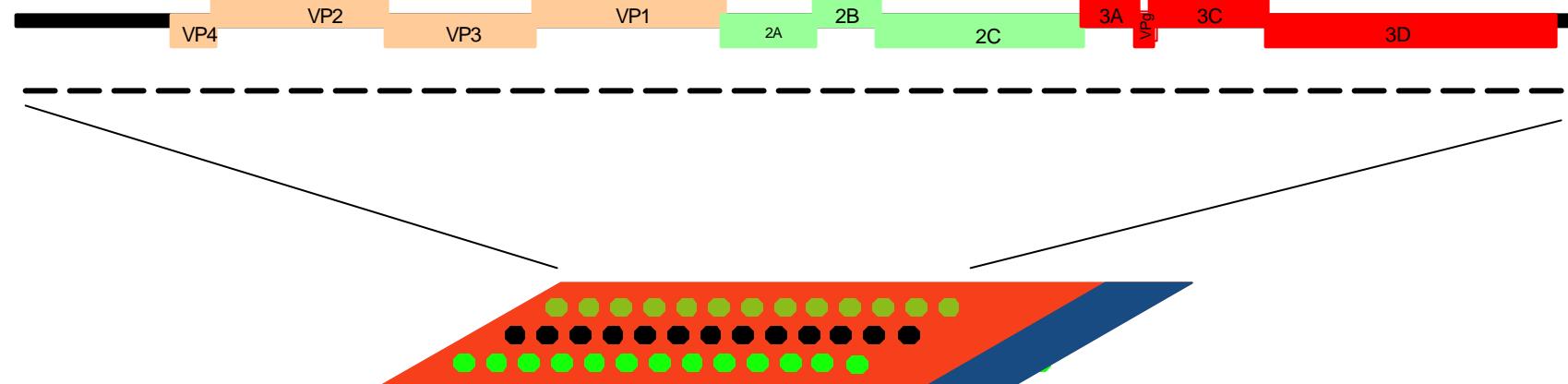
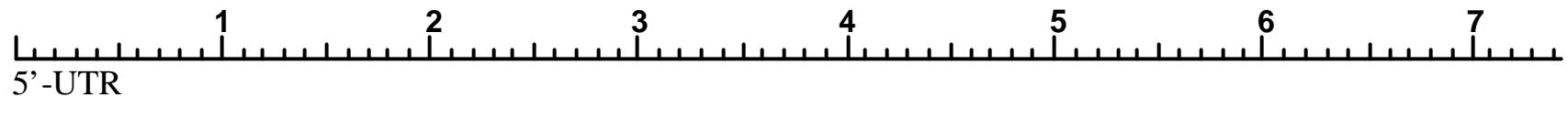




Printing Oligonucleotide Microarrays



Recombinant Vaccine-derived Strains of Poliovirus



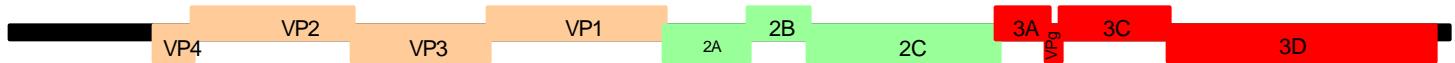
Sabin 1

Sabin 2

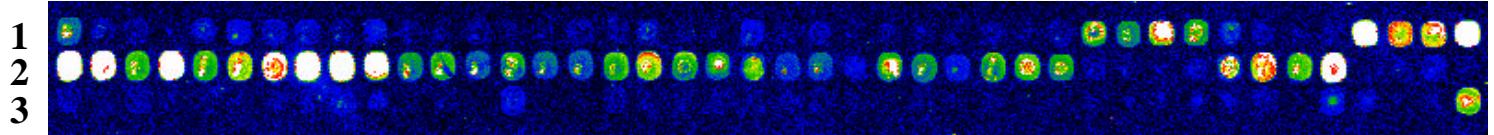
Sabin 3



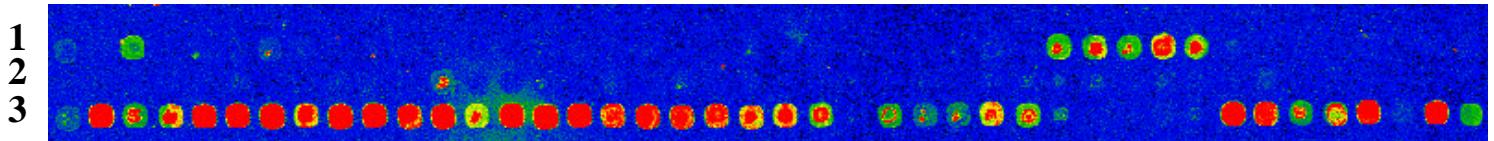
Analysis of Recombinant Vaccine-derived Polioviruses



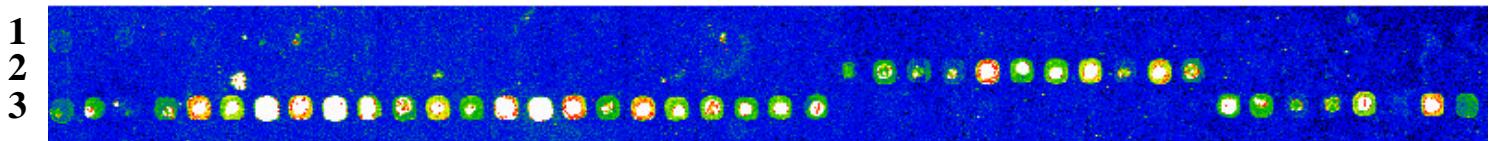
Isolate 9097



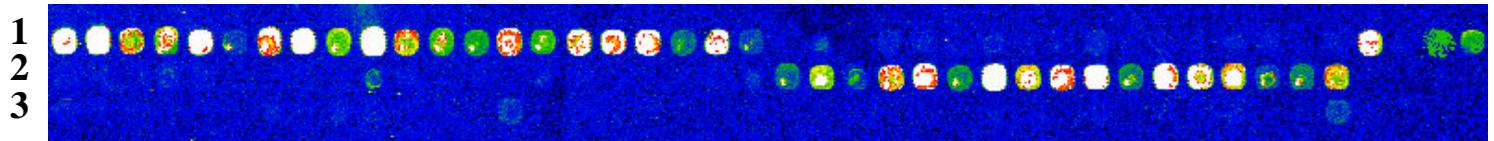
Isolate 7532



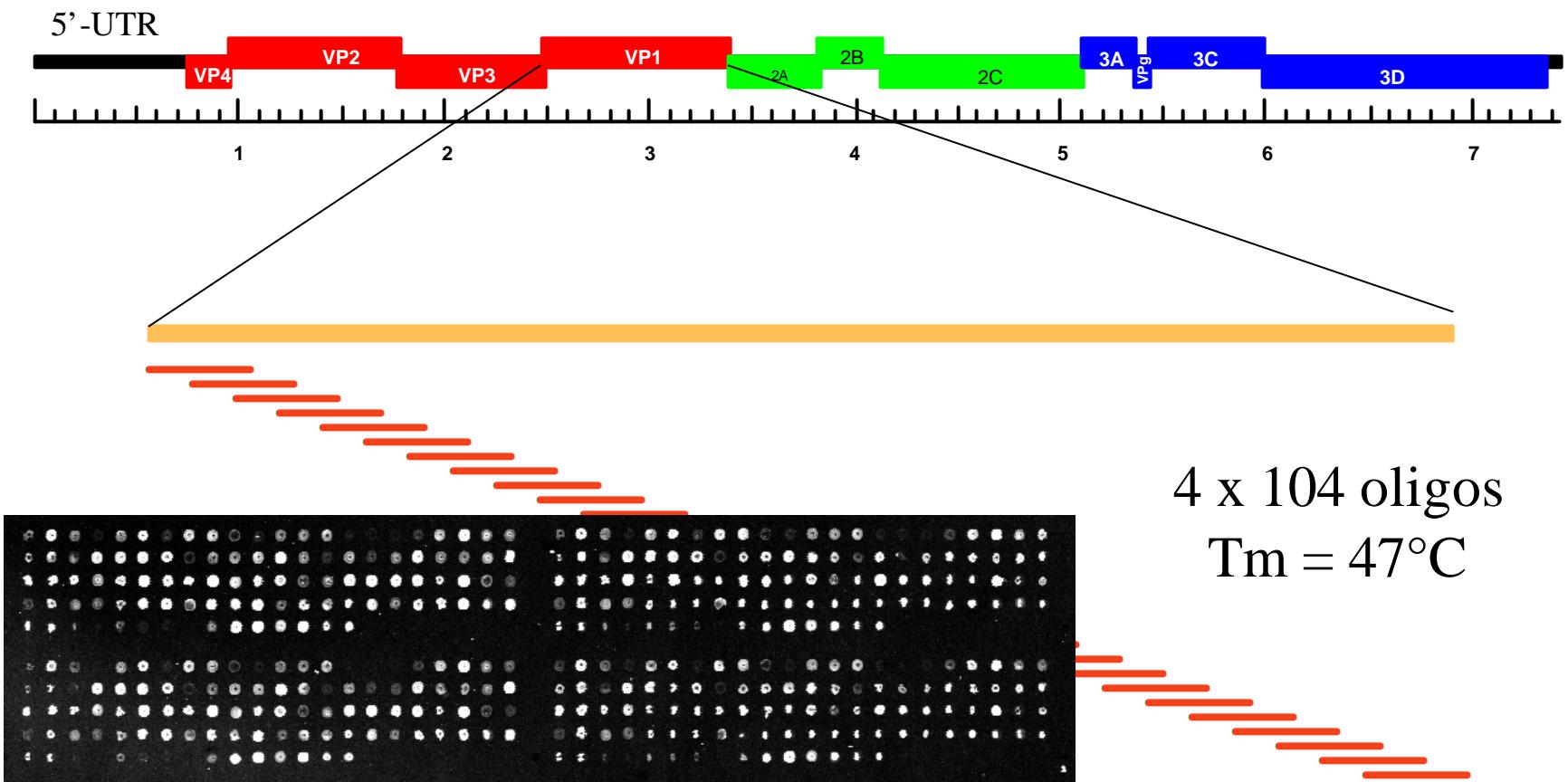
Isolate 6061



Isolate 11262

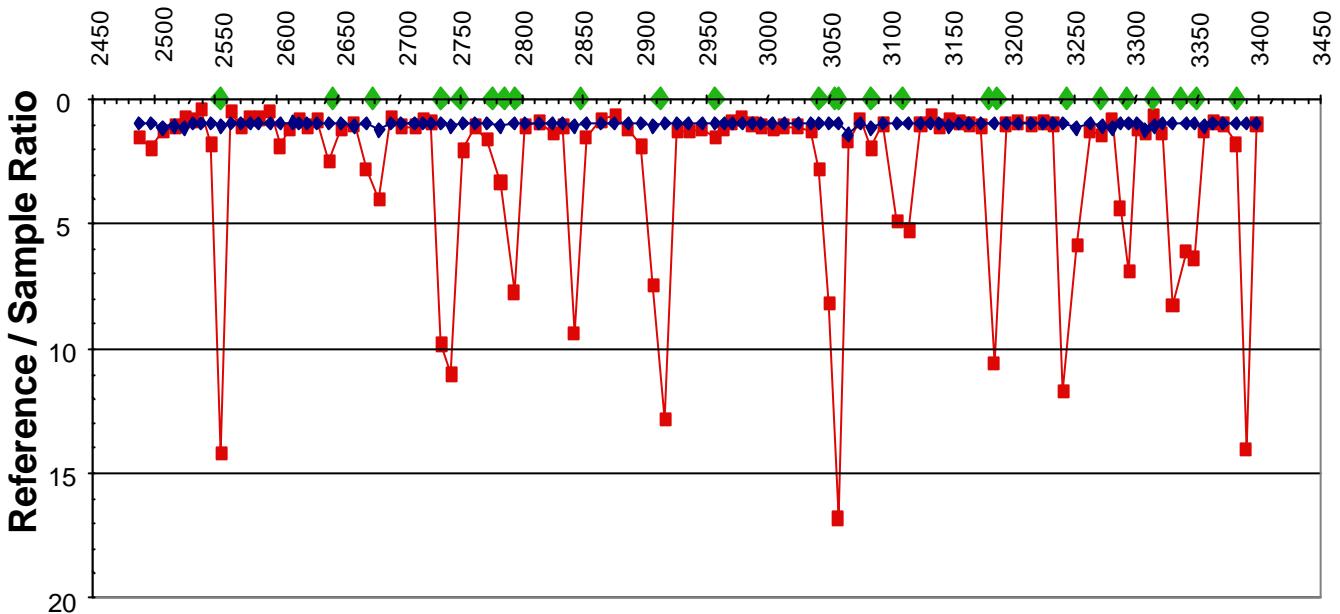


Screening for point mutations: “Sliding oligo” method



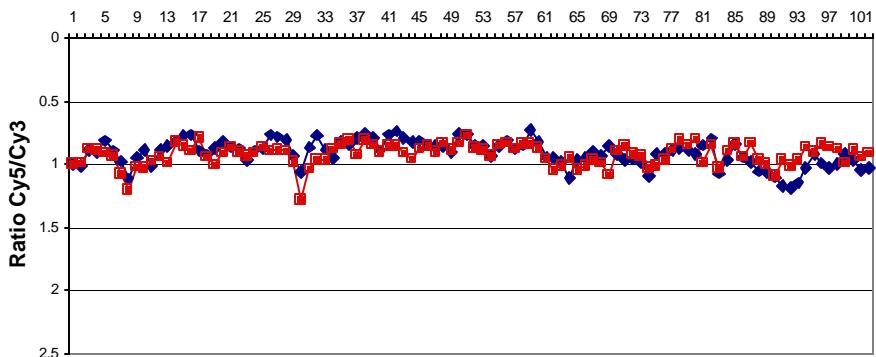


Genetic Drift of Vaccine-Derived Poliovirus Isolates

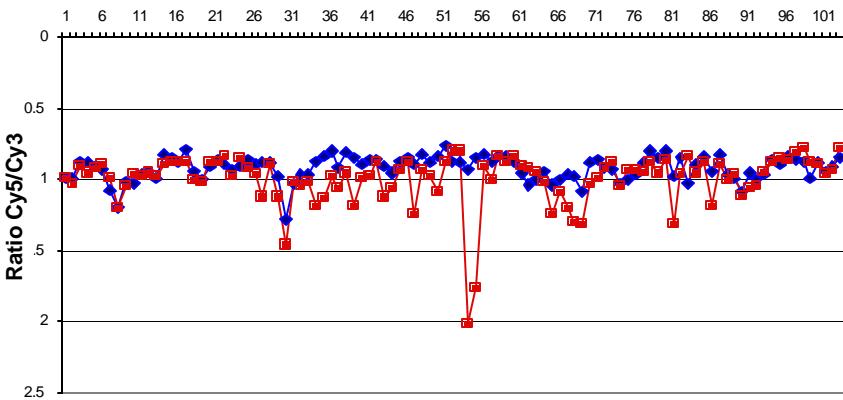


Microheterogeneities in Attenuated Poliovirus

First Passage



Tenth Passage



OP #54 CAGGCGCTCCA **GTGC**

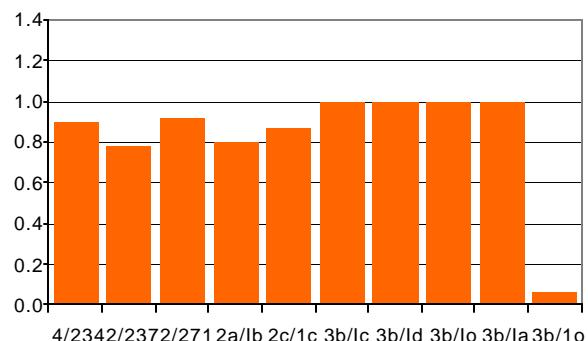
OP #55 TCCA **GTGCCCGAGAAAT**

Vaccine-derived “runaway” polioviruses

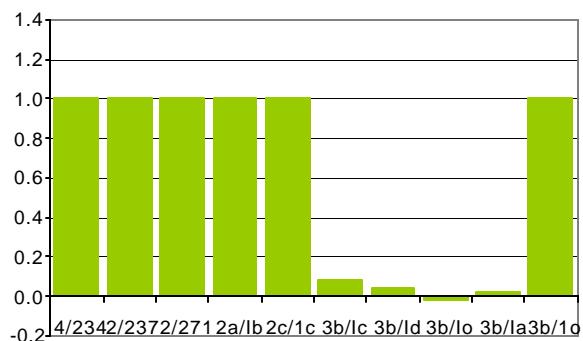
- * Why do they do this?
- * What is common to all of them?

Antigenic Profiles of VDPV

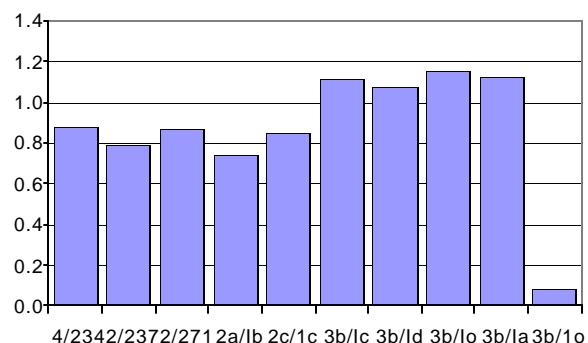
Manone



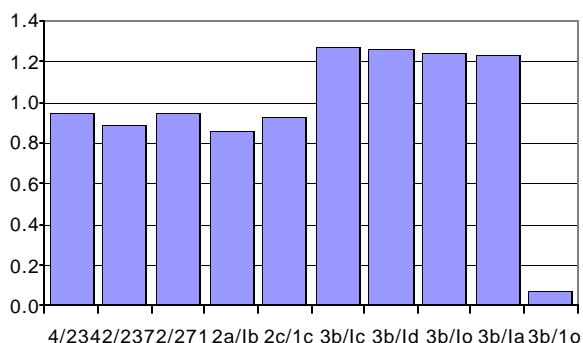
Sabin



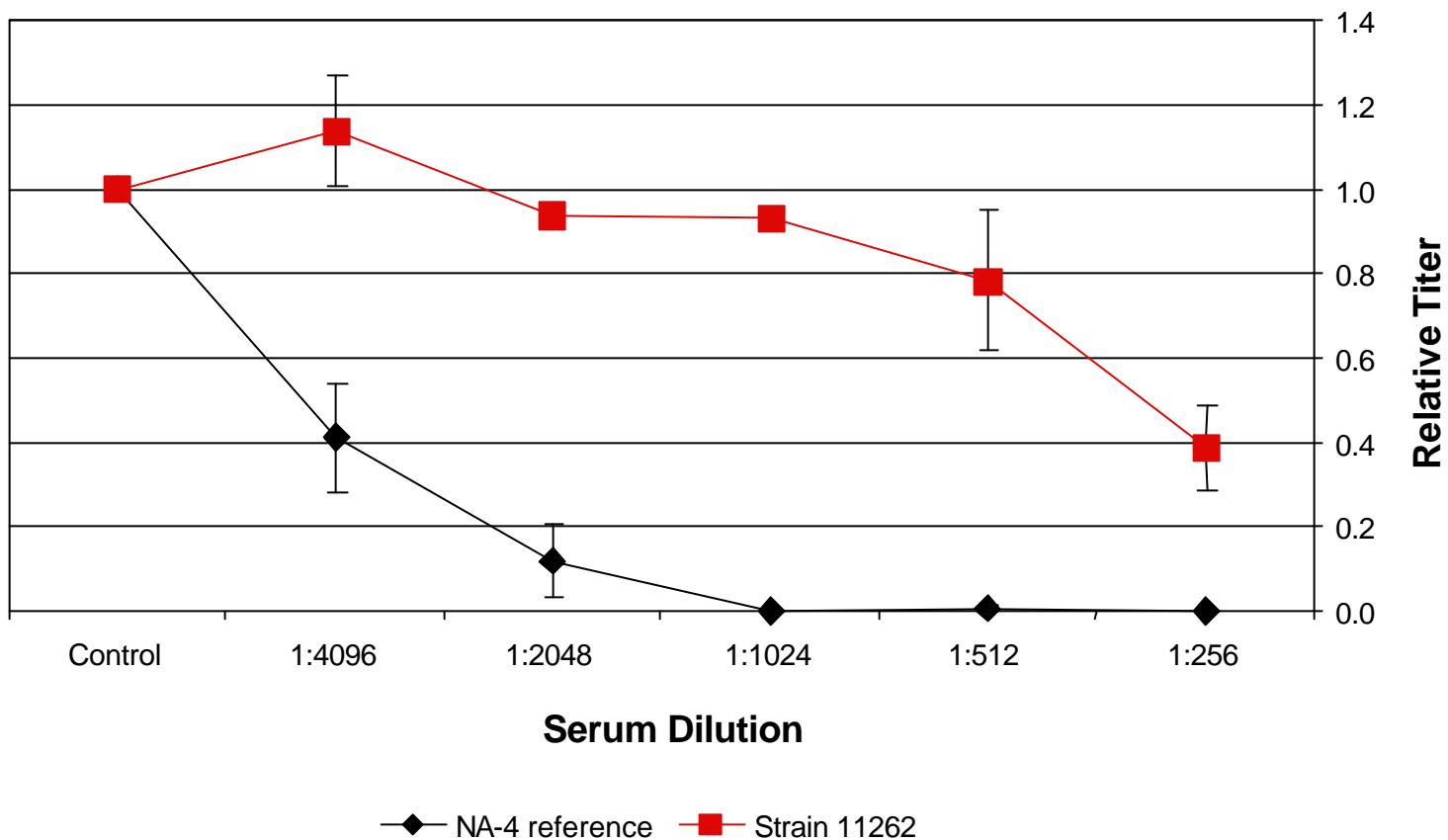
11262



8757



Antigenic Drift as a Driving Force ?



Conclusions

Molecular consistency monitoring helps:

- * Bring transparency to manufacturing process
- * Improve safety of vaccines
- * Surveillance of long-term genetic stability of live vaccines
- * Develop sound vaccination policies
- * Return public confidence in vaccines

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